Reprinted from JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 1995, 43.

Herbivore Avoidance of a Simple Digitalis Extract

Dale L. Nolte,*,† Bruce A. Kimball,‡ Kevin L. Kelly,‡ Zhibin Zhang,§ and Dan L. Campbell†

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, 1835 Black Lake Boulevard, Olympia, Washington 98512, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, Building 16, Denver Federal Center, P.O. Box 25266, Denver, Colorado 80225, and Department of Animal Ecology, Institute of Zoology, Chinese Academy of Science, Beijing, People's Republic of China

Browsing or clipping of seedlings by herbivores hinders reforestation efforts in the Pacific Northwest. Plants that are naturally avoided by herbivores may provide a source of aversive agents to serve as repellents. This study was conducted to determine whether mountain beaver (*Aplodontia rufa*) would ingest apple cubes treated with a simple water extract of digitalis (*Digitalis purpurea*). Responses were assessed in two-choice and one-choice tests. Under both test regimes, mountain beavers reduced ($P < 0.05$) their intake of apple treated with the extract. It was concluded that digitalis may be an effective source of aversive agents to deter herbivore damage in the Pacific Northwest.

Keywords: *Digitalis; herbivore; mountain beaver; repellent*

INTRODUCTION

Browsing or clipping of seedlings by herbivores hinders reforestation efforts in the Pacific Northwest (Black and Lawrence, 1992). Methods available to limit this damage are generally lethal and difficult to implement on a large scale. Public perception and increasing operational expenses further impede the use of traditional technologies. As a consequence, alternative control strategies to minimize damage are needed, and repellents may provide a viable option (Feldhamer and Rochelle, 1982).

Plants naturally avoided by herbivores may be a potential source of aversive agents (Cardellina, 1988). For example, herbivores rarely ingest digitalis (*Digitalis purpurea*; Corrigall et al., 1978). However, it is unknown whether herbivores will avoid palatable plants when these desirable forages are treated with extracts derived from digitalis.

The objective of this study was to determine whether a herbivore would ingest a preferred food treated with a simple water extract of digitalis. Mountain beavers (*Aplodontia rufa*), a fossorial rodent endemic to the Pacific Northwest, were chosen as subjects to model the response. Strictly herbivorous, mountain beavers are rather indiscriminate foragers ingesting some plants avoided by other herbivores (Voth, 1968). Digitalis, however, is generally not ingested by mountain beavers (unpublished data).

MATERIALS AND METHODS

Stimuli. Digitalis was collected from the Capitol State Forest, Grays Harbor County, approximately 30 km from Olympia, WA. Samples were frozen and then lyophilized for 48 h. The lyophilized digitalis and the resultant condensate (RC), approximately 80% of the sample weight, were collected for use as stimuli. Vapors collected in the condenser (−40 °C) during lyophilization primarily result from the sublimation of water. However, sublimation of volatile compounds whose triple point exceeds the conditions of the freeze-dryer will also occur. Vapors may also result from the evaporation of volatile compounds that are present in the liquid state. The highly volatile constituents of digitalis present in RC were thus removed from digitalis by sublimation and condensed with the water. The lyophilized digitalis (dried material) was ground through a No. 20 Wiley mill, then added to water (1 g/2 mL), stirred slowly, and heated (85 °C) for 8 h. Subsequently, solids were removed by pouring the water extract (WE) through a course filter. Therefore, WE consisted of the water-soluble, polar constituents extracted from the plant material following lyophilization. Prior to treatment, RC was also diluted with water (4 g/2 mL). Thus, WE and RC concentrations reflected their respective ratio (1:4) in the fresh digitalis. WE and RC as well as a plain water control (CON) were maintained at 6 °C until applied to the test system. Immediately prior to test, apple cubes (1 cm^3) were submerged in their respective treatments for 1 h.

Subjects. Experimentally naive male (six) and female (six) mountain beavers, trapped in the vicinity where the digitalis was collected, were used in the experiment. Animals were penned individually in outdoor pens (3 × 3 m) and given free access to pelleted feed (X-Cell Feed Co., Tacoma, WA) and water throughout trials. Centrally located nest chambers consisted of three covered plastic cans (76 L) connected with corrugated pipe (30 × 10 cm diameter). Food was available in covered dishes outside the nest area. Experimental animals were given a minimum of 4 days to adjust to captivity and the test regime prior to the onset of trials.

Behavioral Assays. Responses of mountain beavers to treated apple cubes (1 cm^3) were assessed in two-choice and one-choice tests. Two-choice tests are sensitive tests of discrimination between two foods because the subjects can avoid a repellent merely by feeding on the untreated (control) food (Mason et al., 1989). Conversely, one-choice tests are more stringent tests of repellency because the animals must either ingest the treated food or ingest none at all. Apples were selected as the common food among treatments because they are readily ingested by mountain beavers.

Two-Choice Test. Two, two-choice tests were conducted to determine whether mountain beaver differentiated between WE and CON treated apple cubes and between RC and CON treated apple cubes. Trials were counterbalanced; therefore, half of the subjects (three males and three females) were first given a choice between 20 apple cubes treated with WE or 20 apple cubes treated with CON on 4 consecutive days. Conversely, in similar trials, the other subjects (three males and

* Author to whom correspondence should be addressed.
† U.S. Department of Agriculture, Olympia.
‡ U.S. Department of Agriculture, Denver.
§ Chinese Academy of Science.

0021-8561/95/1443-0830$09.00/0

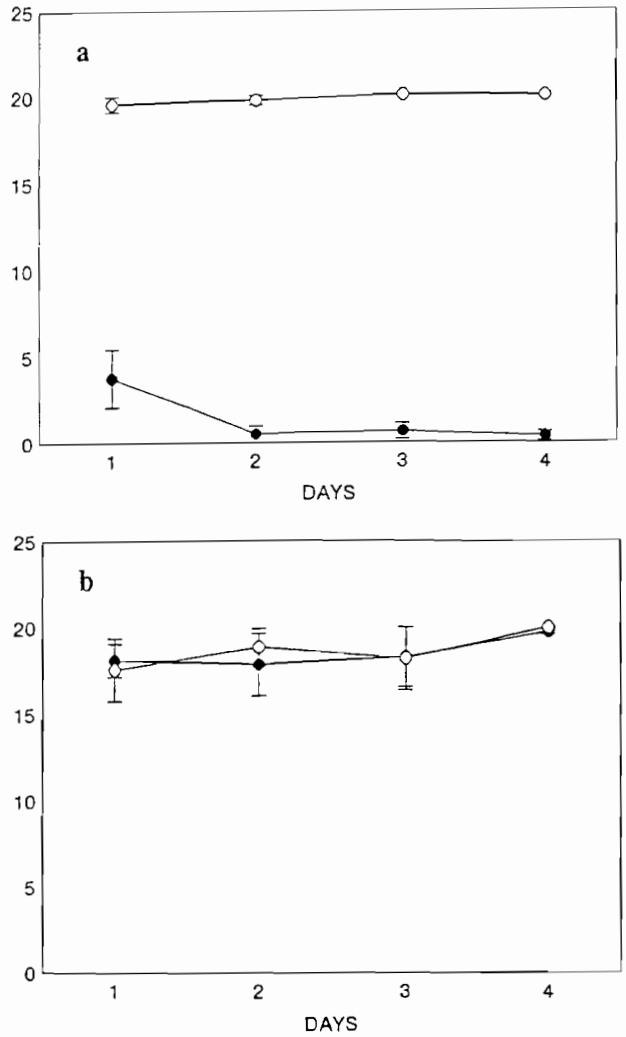

Figure 1. Number of apple cubes taken during a two-choice test when mountain beavers were offered 20 cubes treated with a digitalis water extract (solid circles) and 20 cubes treated with water (open circles) (a) and during a similar test when mountain beavers were offered cubes treated with resultant condensate (solid circles) and cubes treated with water (open circles) (b). Vertical capped bars depict 1 SE.

three females) were first given a choice between RC and CON and then WE and CON. In all cases, apple cubes were provided in the morning at approximately 9:30 a.m. and intake was assessed at 24 h. After 24 h, test foods were removed and replaced with fresh apple cubes with their respective treatments. Location of the CON was randomly selected on the first day and alternated on subsequent days.

One-Choice Test. After a week delay, animals used in the two-choice tests were tested for their acceptance of WE in one-choice tests. On each of 4 consecutive pretreatment days animals were given 20 untreated apple cubes as described above. The number of apple cubes removed was assessed after 24 h, and a fresh set of apple cubes was provided. A 4 day treatment period immediately followed pretreatment. Treatment procedures were similar to those described for pretreatment, except the mountain beavers were presented with 20 WE treated apple cubes on each of the 4 treatment days.

Data Analysis. A four-way repeated-measures analysis of variance (ANOVA) was used to assess differences among experimental factors in the two-choice tests. Between-subject factors were sex (two levels) and order of test (two levels), while within-subject factors were treatment (four levels) and day (four levels) (Winer, 1971). Tukey post hoc tests were used to isolate difference among means ($P < 0.05$).

A three-way ANOVA with repeated measures over periods (two levels) and days (four levels) was used to assess the results of the one-choice tests. Sex was the between-subject factor in the analysis.

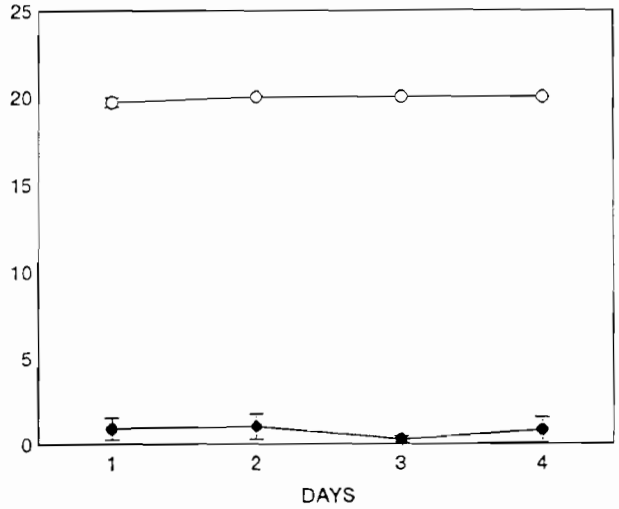

Figure 2. Number of apple cubes taken during a pretreatment period (open circles) when mountain beavers were offered 20 untreated apples cubes and during a subsequent treatment period when they were offered 20 apple cubes treated with a water extract of digitalis (solid circles). Vertical capped bars depict 1 SE.

RESULTS

Intake of apple cubes during the two-choice tests varied among treatments ($P < 0.0001$; Figure 1). Similar numbers of apple cubes were taken irrespective of whether the cubes were treated with RC or water (controls). However, the mountain beavers ingested fewer apple cubes treated with WE ($P < 0.05$). There were no differences between sexes ($P = 0.0922$), in the amount ingested on consecutive days ($P > 0.35$), or in the order in which the tests were administered ($P = 0.3048$). Nor were there any significant interactions ($P > 0.05$).

In the one-choice tests, mountain beavers ingested fewer apple cubes during the treatment period than during the pretreatment period ($P < 0.0001$; Figure 2). Male and female animals responded similarly ($P = 0.2615$), and there were no day effects ($P > 0.35$). No significant interactions occurred ($P > 0.05$).

DISCUSSION

Mountain beavers avoided water extracts of digitalis; however, they readily ingested apple cubes treated with the resultant condensate. These results demonstrate that one or more water-soluble compounds are present in WE at a sufficient concentration to induce food avoidance. Highly volatile compounds that may have been present in the RC either were not aversive to mountain beaver or were not present in sufficient quantities to cause an avoidance.

Repellents inhibit animal foraging by decreasing a plant's palatability. This deterrence may be achieved either through a conditioned aversion or through an unlearned initial avoidance (Mason and Clark, 1992). Conditioned food aversions occur when ingestion of a novel food is paired with nausea (Garcia and Koelling, 1966; Garcia, 1989). Thus, any flavor paired with gastrointestinal distress can become an effective repellent. Repellents that elicit initial avoidance are generally either irritants (e.g., capsaicin) or those that evoke a "fear" response (e.g., predator scents).

Toxins that induce nausea are present in digitalis (Joubert, 1989); however, it is unknown whether the avoidance of digitalis is a learned aversion or an innate response. Extracts in these trials were avoided by mountain beavers on the first day of trials, but these animals had probably encountered digitalis prior to being captured. Regardless, the digitalis cues avoided by animals may be nontoxic. Gustatory or olfactory cues of a food, and the specific compounds that cause aversive feedback, need not be and probably rarely are synonymous (Provenza and Balph, 1990).

Digitalis extracts may provide a viable option to inhibit herbivore damage in the Pacific Northwest. It is difficult to extrapolate these results to other species; however, digitalis is generally avoided by all herbivores. Herbivore familiarity with the plant may be beneficial in the use of digitalis extracts as repellents. Its abundance in the Pacific Northwest ensures that if avoidance requires learning, most animals will recognize its aversive properties through prior encounters. Experiences with the digitalis plant may also reduce habituation to its aversive cues. Animals that ignore cues emitted by the plant risk exposure to digitalis' toxic characteristics.

LITERATURE CITED

Black, H. C.; Lawrence, W. H. Animal Damage Management in Pacific Northwest Forests: 1901–1990. In *Silvicultural Approaches to Animal Damage Management in Pacific Northwest Forest*; Black, H. C., Ed.; U.S. Dept. Agric. For. Ser. PNRS, Gen. Tech. Rep. PNW-GTR-287; Portland, OR, 1992; pp 23–57.

Cardellina, J. H. Natural products in the search for new agrochemicals. In *Biologically Active Natural Products Potential Uses in Agriculture*; Cutler, H. G., Ed.; American Chemical Society: Washington, DC, 1988; pp 305–315.

Corrigall, W.; Moody R. R.; Forbes J. C. Foxglove *Digitalis purpurea* poisoning in farmed red deer *Cervus elaphus*. *Vet. Rec.* **1978**, *102*, 119–122.

Feldhamer, G. A.; Rochelle, J. A. Mountain beaver—*Aplodontia rufa*. In *Wild Mammals of North America*; Chapman, J. A., Feldhamer, G. A., Eds.; John Hopkins University Press: Baltimore, 1982; pp 167–175.

Garcia, J. Food for Tolman: cognition and cathexis in concert. In *Aversion, Avoidance and Anxiety*; Archer, T., Nilson, L., Eds.; Lawrence-Earlbraum: Hillsdale, NJ, 1989; pp 45–85.

Garcia, J.; Koelling, R. A. Relation of cue consequence in avoidance learning. *Psychon. Sci.* **1966**, *4*, 123–124.

Joubert, J. P. J. Cardiac glycosides. In *Toxicants of Plant Origin, Volume II: Glycosides*; Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; pp 61–96.

Mason, J. R.; Clark, L. Nonlethal repellents: the development of cost-effective, practical solutions to agricultural and industrial problems. *Proc. Vertebr. Pest Conf.* **1992**, *15*, 115–129.

Mason, J. R.; Adams, M. A.; Clark, L. Anthranilate repellency to starlings: chemical correlates and sensory perception. *J. Wildl. Manage.* **1989**, *53*, 55–64.

Provenza, F. D.; Balphs, D. F. Applicability of five diet-selection models to various foraging challenges ruminants encounter. In *Behavioral Mechanisms of Food Selection*; Hughes, R. N., Ed.; NATO ASI Series Vol. G 20; Springer-Verlag: Berlin, 1990; pp 423–459.

Voth, E. H. Food Habits of the Pacific Mountain Beaver *Aplodontia rufa pacifica*. Ph.D. Dissertation, Oregon State University, Corvallis, OR, 1968; 263 pp.

Winer, B. G. *Statistical Principles in Experimental Design*; McGraw-Hill: New York, 1971; 907 pp.

Received for review July 21, 1994. Revised manuscript received November 1, 1994. Accepted November 17, 1994.[⊗]

JF940414+

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1995.